

TITLEPLANT VIRAL MOVEMENT PROTEIN GENES.

This application claims the benefit of U.S. Provisional Application No. 60/128,092, filed April 7, 1999.

FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding viral movement proteins in plants and seeds.

BACKGROUND OF THE INVENTION

The phloem of a plant is a vascular tissue that is responsible for distributing the products of photosynthesis, nutrients and hormones to plant tissues and organs. Associated with the phloem are sieve elements and companion cells. Mature sieve cells are enucleate and must rely on physically connected companion cells (via a branched plasmodesmata) to provide many physiological functions. Sieve cells and companion cells together serve to deliver proteins into the phloem. Research has shown that specific mRNA molecules can be found in the plasmodesmata suggesting that there are mechanisms that participate in mRNA transport through the sieve cell-companion cell plasmodesmata connection (Xoconostle-Cazares, B., et al., (1999) *Science* 283:94-98). Some plant viruses have been shown to be able to establish systemic infections via movement proteins (MP) that have the capacity to interact with the plasmodesmata and foster the cell-cell transport of MP and viral nucleic acids. Thus plant viruses have evolved the capacity to utilize existing plant pathways to traffic macromolecules to surrounding cells. Plants appear to have proteins similar to viral movement proteins that function in the transport of nucleic acids from cell to cell. Several plant genes that encode viral movement protein homologs have been identified in rice (elicitor-responsive gene 3, Os-FIERG1 and Os-FIERG2), while one has been identified in corn (novel gene) and one has been identified in *Cucurbita maxima* (CmPP16) (Xoconostle-Cazares, B., et al., (1999) *Science* 283:94-98). Interestingly, movement of RNA throughout the plant is postulated by some to explain the phenomena of cosuppression. Thus, understanding plant viral movement protein homologs and how they work will provide mechanisms to control cosuppression and provide mechanisms to engineer plant virus resistance.

SUMMARY OF THE INVENTION

The present invention concerns an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of: (a) a first nucleotide sequence encoding a polypeptide of at least 129 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 32, or (b) a second nucleotide sequence comprising the complement of the first nucleotide sequence.

In a second embodiment, it is preferred that the isolated polynucleotide of the claimed invention comprises a first nucleotide sequence which comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 and 31 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 32.

In a third embodiment, this invention concerns an isolated polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 and 31 and the complement of such nucleotide sequences.

In a fourth embodiment, this invention relates to a chimeric gene comprising an isolated polynucleotide of the present invention operably linked to at least one suitable regulatory sequence.

In a fifth embodiment, the present invention concerns a host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention. The host cell may be eukaryotic, such as a yeast or a plant cell, or prokaryotic, such as a bacterial cell. The present invention also relates to a virus, preferably a baculovirus, comprising an isolated polynucleotide of the present invention or a chimeric gene of the present invention.

In a sixth embodiment, the invention also relates to a process for producing a host cell comprising a chimeric gene of the present invention or an isolated polynucleotide of the present invention, the process comprising either transforming or transfecting a compatible host cell with a chimeric gene or isolated polynucleotide of the present invention.

In a seventh embodiment, the invention concerns a viral movement protein of at least 129 amino acids comprising at least 95% identity based on the Clustal method of alignment compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 32.

In an eighth embodiment, the invention relates to a method of selecting an isolated polynucleotide that affects the level of expression of a viral movement protein or enzyme activity in a host cell, preferably a plant cell, the method comprising the steps of:

(a) constructing an isolated polynucleotide of the present invention or a chimeric gene of the present invention; (b) introducing the isolated polynucleotide or the chimeric gene into a host cell; (c) measuring the level of the viral movement proteins polypeptide or enzyme activity in the host cell containing the isolated polynucleotide; and (d) comparing the level of the viral movement protein or enzyme activity in the host cell containing the isolated polynucleotide with the level of the viral movement protein or enzyme activity in the host cell that does not contain the isolated polynucleotide.

In a ninth embodiment, the invention concerns a method of obtaining a nucleic acid fragment encoding a substantial portion of a viral movement protein, preferably a plant viral

movement protein, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 and 31 and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a viral movement protein amino acid sequence.

In a tenth embodiment, this invention relates to a method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding a viral movement protein comprising the steps of: probing a cDNA or genomic library with an isolated polynucleotide of the present invention; identifying a DNA clone that hybridizes with an isolated polynucleotide of the present invention; isolating the identified DNA clone; and sequencing the cDNA or genomic fragment that comprises the isolated DNA clone.

In an eleventh embodiment, this invention concerns a composition, such as a hybridization mixture, comprising an isolated polynucleotide of the present invention.

In a twelfth embodiment, this invention concerns a method for positive selection of a transformed cell comprising: (a) transforming a host cell with the chimeric gene of the present invention or a construct of the present invention; and (b) growing the transformed host cell, preferably a plant cell, such as a monocot or a dicot, under conditions which allow expression of the viral movement protein polynucleotide in an amount sufficient to complement a null mutant to provide a positive selection means.

BRIEF DESCRIPTION OF THE SEQUENCE LISTINGS

The invention can be more fully understood from the following detailed description and the accompanying Sequence Listing which form a part of this application.

Table 1 lists the polypeptides that are described herein, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. Table 1 also identifies the cDNA clones as individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), contigs assembled from two or more ESTs ("Contig"), contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding the entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"). Nucleotide sequences, SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 and 31 and amino acid sequences SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 32 were determined by further sequence analysis of cDNA clones encoding the amino acid sequences set forth in SEQ ID NOs:34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54 and 56. Nucleotide SEQ ID NOs:31, 35, 37, 39, 41, 43, 45, 47, 49, 51, 52, 53 and 55 and amino acid SEQ ID NOs:34, 36, 38, 40,

42, 44, 46, 48, 50, 52, 54 and 56 were among those disclosed in a U.S. Provisional Application No. 60/128,092, filed April 7, 1999.

The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

TABLE 1
Viral Movement Proteins

Protein	Clone Designation	SEQ ID NO:	
		(Nucleotide)	(Amino Acid)
Viral Movement Protein	vp11c.pk004.d6	1	2
Viral Movement Protein	cta1n.pk0056.d7 (CGS)	3	4
Viral Movement Protein	cta1n.pk0070.g5 (CGS)	5	6
Viral Movement Protein	Contig (CGS) composed of: ehb2c.pk007.b10 ehb2c.pk008.c17 ehb2c.pk012.h20 ehb2c.pk017.o18	7	8
Viral Movement Protein	wr1.pk151.c12 (CGS)	9	10
Viral Movement Protein	rr1.pk087.f5 (CGS)	11	12
Viral Movement Protein	src3c.pk024.h11 (CGS)	13	14
Viral Movement Protein	p0010.cbpcf32r (CGS)	15	16
Viral Movement Protein	ehb1c.pk001.a20 (EST)	17	18
Viral Movement Protein	sls2c.pk011.d4 (CGS)	19	20
Viral Movement Protein	src2c.pk005.o15 (CGS)	21	22
Viral Movement Protein	wlm96.pk039.k12 (CGS)	23	24
Viral Movement Protein	rsl1n.pk010.i2 (FIS)	25	26
Viral Movement Protein	rdr1f.pk001.g6 (CGS)	27	28
Viral Movement Protein	sls1c.pk023.c9 (CGS)	29	30
Viral Movement Protein	wre1n.pk0035.f6 (CGS)	31	32
Viral Movement Protein	Contig composed of: cta1n.pk0056.d7 (EST) p0058.chpbn09r (EST)	33	34
Viral Movement Protein	cta1n.pk0070.g5 (EST)	35	36
Viral Movement Protein	wr1.pk151.c12 (EST)	37	38
Viral Movement Protein	rr1.pk087.f5 (EST)	39	40
Viral Movement Protein	Contig composed of: src2c.pk015.m1 src3c.pk024.h11 (EST)	41	42
Viral Movement Protein	p0010.cbpcf32r (EST)	43	44
Viral Movement Protein	src2c.pk005.o15 (EST)	45	46

Protein	Clone Designation	SEQ ID NO:	
		(Nucleotide)	(Amino Acid)
Viral Movement Protein	wlm96.pk039.k12 (EST)	47	48
Viral Movement Protein	rsl1n.pk010.i2 (EST)	49	50
Viral Movement Protein	rdr1f.pk001.g6 (EST)	51	52
Viral Movement Protein	sls1c.pk023.c9 (EST)	53	54
Viral Movement Protein	wre1n.pk0035.f6 (EST)	55	56

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Res.* 13:3021-3030 (1985) and in the *Biochemical J.* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. The terms "polynucleotide", "polynucleotide sequence", "nucleic acid sequence", and "nucleic acid fragment"/"isolated nucleic acid fragment" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least one of 60 contiguous nucleotides, preferably at least one of 40 contiguous nucleotides, most preferably one of at least 30 contiguous nucleotides derived from SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 and 31 or the complement of such sequences.

The term "isolated polynucleotide" refers to a polynucleotide that is substantially free from other nucleic acid sequences, such as and not limited to other chromosomal and extrachromosomal DNA and RNA. Isolated polynucleotides may be purified from a host cell in which they naturally occur. Conventional nucleic acid purification methods known to skilled artisans may be used to obtain isolated polynucleotides. The term also embraces recombinant polynucleotides and chemically synthesized polynucleotides.

The term "recombinant" means, for example, that a nucleic acid sequence is made by an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated nucleic acids by genetic engineering techniques.

As used herein, "contig" refers to a nucleotide sequence that is assembled from two or more constituent nucleotide sequences that share common or overlapping regions of

sequence homology. For example, the nucleotide sequences of two or more nucleic acid fragments can be compared and aligned in order to identify common or overlapping sequences. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences (and thus their corresponding nucleic acid fragments) can be assembled into a single contiguous nucleotide sequence.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by gene silencing through for example antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting transcript vis-à-vis the ability to mediate gene silencing or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof. The terms "substantially similar" and "corresponding substantially" are used interchangeably herein.

Substantially similar nucleic acid fragments may be selected by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the polypeptide encoded by the unmodified nucleic acid fragment in a plant or plant cell. For example, a substantially similar nucleic acid fragment representing at least one of 30 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a plant or plant cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a plant or plant cell exposed to the substantially similar nucleic fragment can then be compared to the level of the polypeptide in a plant or plant cell that is not exposed to the substantially similar nucleic acid fragment.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by using nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a

more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Consequently, an isolated polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 and 31 and the complement of such nucleotide sequences may be used in methods of selecting an isolated polynucleotide that affects the expression of a viral movement protein in a host cell. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide in a virus or in a host cell (eukaryotic, such as plant or yeast, prokaryotic such as bacterial) may comprise the steps of: constructing an isolated polynucleotide of the present invention or a chimeric gene of the present invention; introducing the isolated polynucleotide or the chimeric gene into a host cell; measuring the level of a polypeptide or enzyme activity in the host cell containing the isolated polynucleotide; and comparing the level of a polypeptide or enzyme activity in the host cell containing the isolated polynucleotide with the level of a polypeptide or enzyme activity in a host cell that does not contain the isolated polynucleotide.

Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) *Nucleic Acid Hybridisation*, IRL Press, Oxford, U.K.). Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C.

Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms commonly employed by those skilled in this art. Suitable nucleic acid fragments (isolated polynucleotides of the present invention) encode polypeptides that are at least about 70% identical, preferably at least about 80% identical to the amino acid sequences reported herein. Preferred nucleic acid fragments encode amino acid sequences that are about 85% identical to the amino acid sequences reported herein. More preferred nucleic acid fragments encode amino acid sequences that are at least about 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are at least about 95% identical to the amino acid sequences reported herein. Suitable nucleic acid fragments not only have the above identities but typically encode a polypeptide having at least 50 amino acids, preferably at least 100 amino acids, more preferably at least 150 amino acids, still more preferably at least 200 amino acids, and most preferably at least 250 amino acids. Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises. Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also www.ncbi.nlm.nih.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant

specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention
5 comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

“Codon degeneracy” refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment
10 comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the “codon-bias” exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage
15 approaches the frequency of preferred codon usage of the host cell.

“Synthetic nucleic acid fragments” can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment.
20 “Chemically synthesized”, as related to a nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid fragments may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization
25 of the nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

“Gene” refers to a nucleic acid fragment that expresses a specific protein, including
30 regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. “Native gene” refers to a gene as found in nature with its own regulatory sequences. “Chimeric gene” refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that
35 are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. “Endogenous gene” refers to a native gene in its natural location in the genome of an organism. A “foreign gene” refers to a gene not normally found in the host organism, but

that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

5 "Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

10 "Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence which can stimulate promoter activity and may be an innate element of
15 the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at
20 different stages of development, or in response to different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82.
25 It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

"Translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is
30 present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol.* 3:225-236).

35 "3' non-coding sequences" refers to nucleotide sequences located downstream of a coding sequence and includes polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid

tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al. (1989) *Plant Cell* 1:671-680.

“RNA transcript” refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. “Messenger RNA (mRNA)” refers to the RNA that is without introns and that can be translated into polypeptides by the cell. “cDNA” refers to DNA that is complementary to and derived from an mRNA template. The cDNA can be single-stranded or converted to double stranded form using, for example, the Klenow fragment of DNA polymerase I. “Sense RNA” refers to an RNA transcript that includes the mRNA and so can be translated into a polypeptide by the cell. “Antisense RNA” refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific nucleotide sequence, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. “Functional RNA” refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term “operably linked” refers to the association of two or more nucleic acid fragments so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term “expression”, as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. “Expression” may also refer to translation of mRNA into a polypeptide. “Antisense inhibition” refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. “Overexpression” refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. “Co-suppression” refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

A “protein” or “polypeptide” is a chain of amino acids arranged in a specific order determined by the coding sequence in a polynucleotide encoding the polypeptide. Each protein or polypeptide has a unique function.

"Altered levels" or "altered expression" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

5 "Null mutant" refers to a host cell which either lacks the expression of a certain polypeptide or expresses a polypeptide which is inactive or does not have any detectable expected enzymatic function.

"Mature protein" refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor protein" refers to the primary product of translation of mRNA; i.e., with pre- and
10 propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. "Chloroplast transit sequence" refers to a
15 nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*)
20 may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys.* 100:1627-1632).

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the
25 transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or "gene gun" transformation technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference). Thus, isolated polynucleotides of the present invention
30 can be incorporated into recombinant constructs, typically DNA constructs, capable of introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described
35 in, e.g., Pouwels et al., *Cloning Vectors: A Laboratory Manual*, 1985, supp. 1987; Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989; and Flevin et al., *Plant Molecular Biology Manual*, Kluwer Academic Publishers, 1990. Typically, plant expression vectors include, for example, one or more cloned plant genes

under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a
5 ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989
10 (hereinafter "Maniatis").

"PCR" or "polymerase chain reaction" is well known by those skilled in the art as a technique used for the amplification of specific DNA segments (U.S. Patent Nos. 4,683,195 and 4,800,159).

The present invention concerns an isolated polynucleotide comprising a nucleotide
15 sequence selected from the group consisting of: (a) a first nucleotide sequence encoding a polypeptide of at least 129 amino acids having at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 32 or (b) a second nucleotide sequence comprising the complement of the first nucleotide sequence.

20 Preferably, the first nucleotide sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 and 31, that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 32.

Nucleic acid fragments encoding at least a portion of several viral movement proteins
25 have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well
30 known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other viral movement proteins, either as cDNAs or
35 genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known

in the art (Maniatis). Moreover, the entire sequence(s) can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673-5677; Loh et al. (1989) *Science* 243:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least one of 60 (preferably one of at least 40, most preferably one of at least 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 and 31 and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide.

The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a viral movement protein, preferably a substantial portion of a plant viral movement protein polypeptide, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 60 (preferably at least one of 40, most preferably at least one of 30) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 and 31 and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using

the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a viral movement protein.

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol.* 36:1-34; Maniatis).

In another embodiment, this invention concerns viruses and host cells comprising either the chimeric genes of the invention as described herein or an isolated polynucleotide of the invention as described herein. Examples of host cells which can be used to practice the invention include, but are not limited to, yeast, bacteria, and plants.

As was noted above, the nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of viral movement-like protein activity in those cells.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. The chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant isolated polynucleotide (or chimeric gene) may be constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al. (1985) *EMBO J.* 4:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics* 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications it may be useful to direct the instant polypeptides to different cellular compartments, or to facilitate secretion from the cell. It is thus envisioned that the

chimeric gene described above may be further supplemented by directing the coding sequence to encode the instant polypeptides with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell* 56:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.* 100:1627-1632) with or without removing targeting sequences that are already present. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of use may be discovered in the future.

It may also be desirable to reduce or eliminate expression of genes encoding the instant polypeptides in plants for some applications. In order to accomplish this, a chimeric gene designed for co-suppression of the instant polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences. Alternatively, a chimeric gene designed to express antisense RNA for all or part of the instant nucleic acid fragment can be constructed by linking the gene or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U.S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or cosuppression construct would act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of gene activity these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a breeding perspective. In addition, the ability to restrict the expression of a specific phenotype to the reproductive tissues of the plant by the use of tissue specific promoters may confer agronomic advantages relative to conventional mutations which may have an effect in all tissues in which a mutant gene is ordinarily expressed.

The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different chimeric genes utilizing different regulatory elements known to the skilled artisan. Once transgenic plants are obtained by one of the methods described above, it will be necessary to screen individual transgenics for those that most effectively display the desired phenotype. Accordingly, the skilled artisan will develop methods for screening large numbers of transformants. The nature of these screens will generally be chosen on practical grounds. For example, one can screen by looking for changes in gene expression

by using antibodies specific for the protein encoded by the gene being suppressed, or one could establish assays that specifically measure enzyme activity. A preferred method will be one which allows large numbers of samples to be processed rapidly, since it will be expected that a large number of transformants will be negative for the desired phenotype.

5 In another embodiment, the present invention concerns a polypeptide of at least 129 amino acids that has at least 95% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 32.

10 The instant polypeptides (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to the proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences
15 that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a chimeric gene for production of the instant polypeptides. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded viral movement protein. An example of a vector for high level expression of the instant polypeptides in a bacterial
20 host is provided (Example 6).

All or a substantial portion of the polynucleotides of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and used as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. For example, the instant nucleic
25 acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics* 1:174-181) in order to construct a genetic map. In addition, the nucleic
30 acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet.*
35 32:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter* 4:37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology

outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

5 Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; *see* Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

10 In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; *see* Laan et al. (1995) *Genome Res.* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

15 A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med.* 11:95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics* 16:325-332), allele-specific ligation (Landegren et al. (1988) *Science* 241:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res.* 18:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nat. Genet.* 7:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid*
20 *Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the
25 instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger
30 and Benzer (1989) *Proc. Natl. Acad. Sci USA* 86:9402-9406; Koes et al. (1995) *Proc. Natl. Acad. Sci USA* 92:8149-8153; Bensen et al. (1995) *Plant Cell* 7:75-84). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which
35 Mutator transposons or some other mutation-causing DNA element has been introduced (*see* Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding the instant polypeptides. Alternatively, the instant nucleic acid fragment may be used as a

hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptides can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptides disclosed herein.

EXAMPLES

The present invention is further defined in the following Examples, in which parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosure of each reference set forth herein is incorporated herein by reference in its entirety.

EXAMPLE 1

Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various *Arabidosis*, grape, corn, rubber tree, rice, soybean and wheat tissues were prepared. The characteristics of the libraries are described below.

TABLE 2

cDNA Libraries from *Arabidosis*, Grape, Corn, Rubber Tree, Rice, Soybean and Wheat

Library	Tissue	Clone
cta1n	Corn tassel*	cta1n.pk0056.d7 cta1n.pk0070.g5
ehb1c	Para rubber tree fast bleeding latex tapped in 2nd day of 3 day tapping cycle	ehb1c.pk001.a20
	Para rubber tree latex tapped in 2nd day of 3 day tapping cycle	ehb2c.pk007.b10 ehb2c.pk008.c17 ehb2c.pk012.h20 ehb2c.pk017.o18
p0010	Corn log phase suspension cells treated with A23187 to induce mass apoptosis**	p0010.cbpcf32r
rdr1f	Rice developing root of 10 day old plant	rdr1f.pk001.g6

Library	Tissue	Clone
rr1	Rice root of two week old developing seedling	rr1.pk087.f5
rsl1n	Rice 15 day old seedling*	rsl1n.pk010.i2
sls1c	Soybean Infected With <i>Sclerotinia sclerotiorum</i> Mycelium	sls1c.pk023.c9
sls2c	Soybean Infected With <i>Sclerotinia sclerotiorum</i> Mycelium	sls2c.pk011.d4
src2c	Soybean 8 Day Old Root Infected With Cyst Nematode <i>Heterodera glycenis</i>	src2c.pk005.o15
src3c	Soybean 8 Day Old Root Infected With Cyst Nematode <i>Heterodera glycenis</i>	src3c.pk024.h11
wlm96	Wheat seedlings 96 hours after inoculation with <i>Erysiphe graminis f. sp tritici</i>	wlm96.pk039.k12
wr1	Wheat root from 7 day old seedling	wr1.pk151.c12
wre1n	Wheat root from 7 day old etiolated seedling*	wre1n.pk0035.f6
vpl1c	Grape in vitro plantlets	vpl1c.pk004.d6

*These libraries were normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

**A23187 is commercially available from Calbiochem-Noavbiochem Corp.

5

cDNA libraries may be prepared by any one of many methods available. For example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science* 252:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

EXAMPLE 2

Identification of cDNA Clones

cDNA clones encoding viral movement proteins were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.*

25

215:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet.* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

EXAMPLE 3

Characterization of cDNA Clones Encoding Viral Movement Proteins

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to viral movement proteins from *Oryza sativa* (NCBI Identifier No. gi 3603473), *Arabidopsis thaliana* (NCBI Identifier No. gi 2911047), *Oryza sativa* (NCBI Identifier No. gi 2920839), *Arabidopsis thaliana* (NCBI Identifier No. gi 2911073), *Cicer arietinum* (NCBI Identifier No. gi 3860331) and *Zea mays* (NCBI Identifier No. gi 1498055). Shown in Table 3 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), contigs assembled from two or more ESTs ("Contig"), contigs assembled from an FIS and one or more ESTs ("Contig*"), or sequences encoding the entire protein derived from an FIS, a contig, or an FIS and PCR ("CGS"):

TABLE 3

BLAST Results for Sequences Encoding Polypeptides Homologous to *Oryza sativa*, *Zea mays*, *Cicer arietinum* and *Arabidopsis thaliana* Viral Movement Proteins

Clone	Status	BLAST pLog Score
vpl1c.pk004.d6	EST	52.52 (gi 3603473)
cta1n.pk0056.d7	CGS	57.10 (gi 3603473)
cta1n.pk0070.g5	CGS	62.22 (gi 3603473)

Clone	Status	BLAST pLog Score
Contig composed of: ehb2c.pk007.b10 ehb2c.pk008.c17 ehb2c.pk012.h20 ehb2c.pk017.o18	CGS	46.00 (gi 3603473)
wr1.pk151.c12	CGS	66.00 (gi 3603473)
rr1.pk087.f5	CGS	33.52 (gi 2911047)
src3c.pk024.h11	CGS	39.40 (gi 2911047)
p0010.cbpcf32r	CGS	61.10 (gi 2920839)
ehb1c.pk001.a20	EST	30.10 (gi 2920839)
sls2c.pk011.d4	CGS	34.05 (gi 2920839)
src2c.pk005.o15	CGS	31.30 (gi 2920839)
wlm96.pk039.k12	CGS	61.40 (gi 2920839)
rs11n.pk010.i2	FIS	66.70 (gi 2911073)
rdrlf.pk001.g6	CGS	61.00 (gi 1498055)
sls1c.pk023.c9	CGS	58.30 (gi 3860331)
wre1n.pk0035.f6	CGS	45.00 (gi 1498055)

The data in Table 4 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 and 32 and the *Oryza sativa*, *Zea mays*, *Cicer arietinum* and *Arabidopsis thaliana* sequences.

TABLE 4

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to *Oryza sativa*, *Zea mays*, *Cicer arietinum* and *Arabidopsis thaliana*

SEQ ID NO.	Percent Identity to
2	83% (gi 3603473)
4	89% (gi 3603473)
6	90% (gi 3603473)
8	82% (gi 3603473)
10	92% (gi 3603473)
12	45% (gi 2911047)
14	48% (gi 2911047)
16	84% (gi 2920839)
18	73% (gi 2920839)
20	71% (gi 2920839)
22	70% (gi 2920839)

SEQ ID NO.	Percent Identity to
24	74% (gi 2920839)
26	36% (gi 2911073)
28	91% (gi 1498055)
30	88% (gi 3860331)
32	71% (gi 1498055)

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments, BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of a viral movement protein.

EXAMPLE 4

Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding the instant polypeptides in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb Sall-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-Sall fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence

analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

5 The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al.
10 (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

15 The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat*
20 gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

 The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 µm
25 in diameter) are coated with DNA using the following technique. Ten µg of plasmid DNAs are added to 50 µL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 µL of a 2.5 M solution) and spermidine free base (20 µL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant
30 removed. The particles are resuspended in 200 µL of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 µL of ethanol. An aliquot (5 µL) of the DNA-coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad
35 Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

 For bombardment, the embryogenic tissue is placed on filter paper over agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of

about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains glufosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing glufosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology* 8:833-839).

EXAMPLE 5

Expression of Chimeric Genes in Dicot Cells

A seed-specific construct composed of the promoter and transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin construct includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire construct is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed construct.

Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic

embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule.

5 Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) 327:70-73, U.S. Patent No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used
10 for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al. (1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase
15 gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed construct comprising the phaseolin 5' region, the fragment encoding the instant polypeptides and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 µL of a 60 mg/mL 1 µm gold particle suspension is added (in order): 5 µL
20 DNA (1 µg/µL), 20 µL spermidine (0.1 M), and 50 µL CaCl₂ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 µL 70% ethanol and resuspended in 40 µL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five µL of the DNA-coated gold particles are
25 then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally
30 bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL
35 hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension

cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

EXAMPLE 6

Expression of Chimeric Genes in Microbial Cells

5 The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene* 56:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoR I and Hind III sites in
10 pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region,
15 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% NuSieve GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10 µg/mL ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the
20 agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 µL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector
25 pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing LB media and 100 µg/mL ampicillin. Transformants containing the gene encoding the instant polypeptides
30 are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol.* 189:113-130). Cultures are grown in LB medium
35 containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 µL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM

- DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One μ g of protein from the soluble fraction of the culture can be separated by
- 5 SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

SEQUENCE LISTING

<110> E. I. du Pont de Nemours and Company

<120> Plant Viral Movement Protein Genes

<130> BB1344

<140>

<141>

<150> 60/128,092

<151> 1999-04-07

<160> 56

<170> Microsoft Office 97

<210> 1

<211> 450

<212> DNA

<213> Vitis sp.

<220>

<221> unsure

<222> (445)

<400> 1

```

gggagaaaag gttagaattt tggtttgcat ttggaatctg ttgcaattct caatcagaaa 60
atgcctcaag gaacacttga agtccttctt gtcagtgcga agggctctga gaacactgat 120
tttctctgta acatggatcc ttatgttggt ctcaacttgc gcactcagga gcagaaaagc 180
agtgttgcat caggaaaagg gtctgaccca gaatggaatg aacattttgt attcaccata 240
tctgaaggca tctcagaact caccattaaa ataatggaca gtgatagcgg tagtggtgat 300
gattttgtgg gagaagcaac cattccacta gaggcactct tcacggaagg aagcctggag 360
ccaagcaccg gtacaatgtt gttaaagacc aaggaatatt gtggagagat taaagttggc 420
ctcactttca ctcaaaaggg aaaangtgat                                     450

```

<210> 2

<211> 130

<212> PRT

<213> Vitis sp.

<220>

<221> UNSURE

<222> (129)

<400> 2

```

Met Pro Gln Gly Thr Leu Glu Val Leu Leu Val Ser Ala Lys Gly Leu
  1              5              10              15

```

```

Glu Asn Thr Asp Phe Leu Cys Asn Met Asp Pro Tyr Val Val Leu Thr
      20              25              30

```

```

Cys Arg Thr Gln Glu Gln Lys Ser Ser Val Ala Ser Gly Lys Gly Ser
      35              40              45

```

```

Asp Pro Glu Trp Asn Glu His Phe Val Phe Thr Ile Ser Glu Gly Ile
      50              55              60

```

```

Ser Glu Leu Thr Ile Lys Ile Met Asp Ser Asp Ser Gly Ser Gly Asp

```

65					70					75					80
Asp	Phe	Val	Gly	Glu	Ala	Thr	Ile	Pro	Leu	Glu	Ala	Leu	Phe	Thr	Glu
				85					90					95	
Gly	Ser	Leu	Glu	Pro	Ser	Thr	Gly	Thr	Met	Leu	Leu	Lys	Thr	Lys	Glu
			100					105					110		
Tyr	Cys	Gly	Glu	Ile	Lys	Val	Gly	Leu	Thr	Phe	Thr	Gln	Lys	Gly	Lys
		115					120					125			

Xaa Asp
130

<210>	3
<211>	916
<212>	DNA
<213>	Zea mays

<400>	3						
gcacgagcac	gccgcctcca	tgtgggtggg	gaggcaaacy	cgttcgtcca	tctctgaaac		60
tcaaacgcct	tgtattggag	catactacag	gagtacttct	gtacaaatat	aaatacccct		120
ggcgagttgg	gttgggtcta	tctcgcaatc	gaggcgtttt	ttttctgctt	cgtaagttcg		180
tggtcgatcc	agcgagcgag	cgagcagacc	ggcggctaac	cgcgagggga	gagatggcgc		240
aggggacgct	ggaggtgctt	ctcgtcggag	ccaggggcct	cgagaacacc	gattacctga		300
gcaacatgga	cccctacgcg	cttctgcaat	gtcgtctcca	cgagcagaag	agcagcgctc		360
catctggcaa	aggctgtgaa	cctgagtggg	acgagacctt	cggtttcacc	gtctccgatg		420
gcgcagcaga	gctgttcctc	aagctcctgg	acagtgacgg	tggcactgat	gacgattttg		480
ttgggtgagge	aacgattcct	ctggaagcag	tttacacgga	aggaaacatc	cctccgactg		540
tttacaatgt	tgtgaaagac	gaagaatacc	gcgagagaa	caaagttggc	ctcacgttca		600
ctccagagga	ccagggcttc	tgaggaataa	cttggcgtgt	ggccgctgga	actggaggca		660
gcaggcagtc	gtcttatgat	tcagaagcaa	acgacggatc	gattcccttg	atgtactgca		720
gtccagtgag	cgtgcattcta	caacttgtag	aagaagcctg	caacatgatc	acgggatcct		780
gtactgcate	actctaaagc	ctagctaaaa	ccaccagctc	ctgtacttga	tgccggggcg		840
gatttgtcatg	tactgaaacc	tacaataacg	gtcgcggaac	cccactcttt	gatgttaaaa		900
aaaaa	aaaaaa						916

<210>	4
<211>	129
<212>	PRT
<213>	Zea mays

```
<400>      4  
Met Ala Gln Gly Thr Leu Glu Val Leu Leu Val Gly Ala Arg Gly Leu  
   1                   5                  10                 15  
  
Glu Asn Thr Asp Tyr Leu Ser Asn Met Asp Pro Tyr Ala Leu Leu Gln  
                20                    25                   30  
  
Cys Arg Ser His Glu Gln Lys Ser Ser Val Ala Ser Gly Lys Gly Cys  
        35                     40                      45  
  
Glu Pro Glu Trp Asn Glu Thr Phe Val Phe Thr Val Ser Asp Gly Ala  
    50                            55                        60  
  
Ala Glu Leu Phe Ile Lys Leu Leu Asp Ser Asp Gly Gly Thr Asp Asp  
    65                          70                       75              80  
  
Asp Phe Val Gly Glu Ala Thr Ile Pro Leu Glu Ala Val Tyr Thr Glu  
          85                     90                       95
```

Gly Asn Ile Pro Pro Thr Val Tyr Asn Val Val Lys Asp Glu Glu Tyr
100 105 110

Arg Gly Glu Ile Lys Val Gly Leu Thr Phe Thr Pro Glu Asp Gln Gly
115 120 125

Phe

<210> 5
<211> 876
<212> DNA
<213> Zea mays

<400> 5
gcacgagggt cgttcacgcc acaggcaagg cacagggggt tgtgagggag agcgaggagc 60
ggaggaggac atggtgcaag ggacgctgga agtgctgctc gttggggcca agggcctcga 120
gaacaccgat tacctctgta acatggatcc gtatgcaatt ctcaagtgcc gttcacagga 180
gcagaagagc agtattgcaa ctggaaaagg aactaccctt gaggaggatg aaaactttat 240
cttcaactgtg tctgaccogga caacagactt ggtaatcaag cttatggaca gtgatacagg 300
cacagcagat gactttgttg gtgaagcaac gattccattg gaagcagtgt atactgaaag 360
gagcattcca ccaacactct ataatgttgt gaaagggtgaa aaatactgag gggaaatcaa 420
agttgggtctc acattcactc ctgaggatac tcgccagcgg ggtctcccag aggacttcgg 480
tggtatggaag caatcatctt agagctagat gctttaaggg tgcaccagag cacagcgaca 540
attcatgcgc ttggagcctt cagccgtcga gtacttcattg ctaatgcaga attcattcga 600
tttggcttct tttgattgtt tcagaagaag tgttattagt gaggtttcaac aaaaaatagc 660
tccatattgc tctatatccc gtattggaaa ttctaaggcc gtttgtgatt actgcttaca 720
acaagaagtt ttgcttctag ttcccactac gctttttttt gaagtttttga gtggaacatc 780
tttgtgttca acgtttgggg aggtgtaggc cagtaataac gcaagaaagg aataatttcc 840
cttgacgcaa cattgttttt tgtgatcctt gaaaaa 876

<210> 6
<211> 143
<212> PRT
<213> Zea mays

<400> 6
Met Val His Gly Thr Leu Glu Val Leu Leu Val Gly Ala Lys Gly Leu
1 5 10 15

Glu Asn Thr Asp Tyr Leu Cys Asn Met Asp Pro Tyr Ala Ile Leu Lys
20 25 30

Cys Arg Ser Gln Glu Gln Lys Ser Ser Ile Ala Thr Gly Lys Gly Thr
35 40 45

Thr Pro Glu Trp Asn Glu Asn Phe Ile Phe Thr Val Ser Asp Arg Thr
50 55 60

Thr Asp Leu Val Ile Lys Leu Met Asp Ser Asp Thr Gly Thr Ala Asp
65 70 75 80

Asp Phe Val Gly Glu Ala Thr Ile Pro Leu Glu Ala Val Tyr Thr Glu
85 90 95

Arg Ser Ile Pro Pro Thr Leu Tyr Asn Val Val Lys Gly Glu Lys Tyr
100 105 110

Cys Gly Glu Ile Lys Val Gly Leu Thr Phe Thr Pro Glu Asp Thr Arg

115

120

125

Gln Arg Gly Leu Pro Glu Asp Phe Gly Gly Trp Lys Gln Ser Ser
 130 135 140

<210> 7
 <211> 771
 <212> DNA
 <213> Hevea brasiliensis

<220>
 <221> unsure
 <222> (671)

<220>
 <221> unsure
 <222> (721)

<220>
 <221> unsure
 <222> (752)

<220>
 <221> unsure
 <222> (767)

<220>
 <221> unsure
 <222> (769)

<400> 7
 cttaattttt aaaaacatta ttagccttcc tcgttaatca ttcactctcc ctataagatg 60
 cacagatacc caaagggcag agatccaaga actcatttca atctaaattc ctgttgagtt 120
 ttaagtcttt tctttttcgc tttttggatt caattctggt ccaaaaatgc ctctaggaac 180
 tggtgaagtc ctacttggtg gtgctaaggg tcttgaaaac actgattttc tcaatggcgt 240
 ggacccttat gtgctcctcg cttgccgtac ccaggagcag aaaagcagtg ttgcttcagg 300
 gaaagggagt gaaccagaat ggaatgagaa attctcattt gaggtatcag atggtgacac 360
 agaactcaca ttgaaaatca tggacagtga tgttggtgct gcagatgatt ttgttgaga 420
 agcaaccatt ccccttgagc cattgttttt ggaaggaaac ctcccatcta cggcgtaaa 480
 agttgtcaaa gaacaagaat acaagggaga gattacagtg ggctcacct tcacccaga 540
 ggtagagatg gacaacgtcg gagtggatgg atacgatttt cggttataat attaactagc 600
 atcttggtgt ggaaatggca aggactgctt ttggtttgga gatggcaaaa gagactccgt 660
 ttttaacgtc natgttggtg ttgaaaactt gggttttgat gtttgcaaaa aataccgat 720
 nttgttttaa agaaaccctt tttggggggt tngaaattga atttggnant t 771

<210> 8
 <211> 140
 <212> PRT
 <213> Hevea brasiliensis

<400> 8

Met Pro Leu Gly Thr Val Glu Val Leu Leu Val Gly Ala Lys Gly Leu
 1 5 10 15

Glu Asn Thr Asp Phe Leu Asn Gly Val Asp Pro Tyr Val Val Leu Ala
 20 25 30

Cys Arg Thr Gln Glu Gln Lys Ser Ser Val Ala Ser Gly Lys Gly Ser
 35 40 45

Glu Pro Glu Trp Asn Glu Lys Phe Ser Phe Glu Val Ser Asp Gly Asp
 50 55 60

Thr Glu Leu Thr Leu Lys Ile Met Asp Ser Asp Val Gly Ala Ala Asp
 65 70 75 80

Asp Phe Val Gly Glu Ala Thr Ile Pro Leu Glu Pro Leu Phe Leu Glu
 85 90 95

Gly Asn Leu Pro Ser Thr Ala Tyr Lys Val Val Lys Glu Gln Glu Tyr
 100 105 110

Lys Gly Glu Ile Thr Val Gly Leu Thr Phe Thr Pro Glu Val Glu Met
 115 120 125

Asp Asn Val Gly Val Asp Gly Tyr Asp Phe Arg Leu
 130 135 140

<210> 9

<211> 874

<212> DNA

<213> Triticum aestivum

<400> 9

gcacgaggcc gagctttcca tttttcaact cctagtccta tacatacagc ggaaccccg 60
 ggctcggatc ggatctacag caattagtct cgaccttcag tcgtgccgcc tgctcatcag 120
 catataattc ctgatcgagc gagcgggaga ggaaggcgag atcaggcccg gagagaagat 180
 ggcgcagggg acgctggagg tgctgctcgt gggagccaag ggcctcgaga acaccgacta 240
 cctctgcaac atggaccgt acgcggttct aaaatgcacc tcgcaggagc aaaagagcac 300
 cgctgcctct ggaaaggga gtgatcctga gtggaacgaa acctttgtgt tcaccgtctc 360
 tgagaatgca actgagcttg tcatcaagct actggacagt gatggtggca cggacgacga 420
 cagcgttggt gaagcaacga tcccattgga tggagtgtac actgaaggaa gcatcccacc 480
 aactgtttac aatgttgtca aagacgaaga gtaccgtgga gaaatcaaaa ttggtctgac 540
 gttcactccg gaggaggctc gtgatcagga tcaacccgag gaaaactatg gtgggtggaa 600
 ccaatcatct tgagaagaag caggtgcttt gctgaactat ggtgcgtgac aagtcgtgtg 660
 ctagaactaa agcctatttt aattgtttaa gactgtattt gtcgttgatt cctcaatta 720
 tggataagct acgaatctac ttattgattg gtatcgtttt ctaatattca aatttgtaat 780
 aacagtgttc ccacttgta tgaagtatga gcctctttaa tgtcactaaa ctgagttgca 840
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaa 874

<210> 10

<211> 144

<212> PRT

<213> Triticum aestivum

<400> 10

Met Ala Gln Gly Thr Leu Glu Val Leu Leu Val Gly Ala Lys Gly Leu
 1 5 10 15

Glu Asn Thr Asp Tyr Leu Cys Asn Met Asp Pro Tyr Ala Val Leu Lys
20 25 30

Cys Thr Ser Gln Glu Gln Lys Ser Thr Val Ala Ser Gly Lys Gly Ser
35 40 45

Asp Pro Glu Trp Asn Glu Thr Phe Val Phe Thr Val Ser Glu Asn Ala
50 55 60

Thr Glu Leu Val Ile Lys Leu Leu Asp Ser Asp Gly Gly Thr Asp Asp
65 70 75 80

Asp Ser Val Gly Glu Ala Thr Ile Pro Leu Asp Gly Val Tyr Thr Glu
85 90 95

Gly Ser Ile Pro Pro Thr Val Tyr Asn Val Val Lys Asp Glu Glu Tyr
100 105 110

Arg Gly Glu Ile Lys Ile Gly Leu Thr Phe Thr Pro Glu Glu Ala Arg
115 120 125

Asp Gln Asp Gln Pro Glu Glu Asn Tyr Gly Gly Trp Asn Gln Ser Ser
130 135 140

<210> 11

<211> 1482

<212> DNA

<213> Oryza sativa

<400> 11

```

gcacgagatc gtcaactcag ctctctctctt tcttcccttc ccccgctcct ccgcgagacg 60
accgcgccc gtagccatcc atgtcgatac aaggccagat cctcgaagtc agagtcaactg 120
ggtgcaggaa gctgagggac acggagttct tcacgcggca ggatccctac gtctgcatcg 180
agtatgccac caacaagttc cgcacccgca cctgcaccga tgggggaagg aaccctactt 240
ttgacgagaa gtttcatata cctctcattg aggggcttcg tgagctaacc gtcacagtgt 300
ggaacagcaa cacgctcacc catgatgatt tcattggcaa tggcaggggtg cagctgcata 360
aggtgcttac gcgtaggctat gatgatgcct catggcccct ccagacacgc catatgaggt 420
ctgctgggga agtgacgctc attatgcatt ttgatgtttc agcaatgaag aacaagccgg 480
gaaaaatttc tgccgcgtca accacacatt ctgttcttcc agtgccggta ccagcagtac 540
catatgctgc cccctcacct tcatacgcac taccctctgc aggataccct gcagtaccgc 600
catatcaatc ctatcctgct agccatgtcc cggcgccata tcctacttca gcataccac 660
atccaccacc atctctgcta gctcgcgatg ttgagcatgc ggcataccct cctacaagta 720
caacatatcc tccacagccg taccacacc agccgcaggg acaaacatac ccaccgcagc 780
cgcagggaga aacataccaa ccgcagccgc agcgagaaac ataccaccgc cagcctcaag 840
tacaaccata cccaccaaag ccacagggac aaccataccc accgcagccg cagggacaac 900
catatccacc gcaaccatat ggacaaactt acccaccacc tccaaaagga cagccacat 960
atccacctgc gccctatcct tcaacttacc caccagcacc atattgatat ggcacacttg 1020
gtggactgaa gttgtccaca tacaaaagca agtaagcaac aagtgatgat cagttcttat 1080
atztatccag ggtatccagc cttcatcatc cagttaattg aaacaaatga aatcattcct 1140
gaagcgattc atgtcaacat cttagcaacc aatggtagta gttaccatct ggtatgtatc 1200
atatatcata gcttgagaa tgtcacgaat ggaatttggt cgattatggt gtatgttttg 1260
ggcttggtgt aacagtgatc cacctttggt ctgttttgag gtcattgttg gctgttctgt 1320
gactgtaact actgcttttt acaaaggggg gaagcagtaa ttctagtctt acctgcaact 1380
gctgataag tgttaactgt gaaaagttgc agtagcttgt cgactttgta ccatgttggt 1440
tgagatgctc aataaatttg ctttgtacta aaaaaaaaaa aa 1482

```

<210> 12

<211> 308

<212> PRT

<213> Oryza sativa

<400> 12

```

Met Ser Ile Gln Gly Gln Ile Leu Glu Val Arg Val Thr Gly Cys Arg
 1           5           10           15

Lys Leu Arg Asp Thr Glu Phe Phe Thr Arg Gln Asp Pro Tyr Val Cys
           20           25           30

Ile Glu Tyr Ala Thr Asn Lys Phe Arg Thr Arg Thr Cys Thr Asp Gly
           35           40           45

Gly Arg Asn Pro Thr Phe Asp Glu Lys Phe His Ile Pro Leu Ile Glu
           50           55           60

Gly Leu Arg Glu Leu Thr Val Thr Val Trp Asn Ser Asn Thr Leu Thr
           65           70           75           80

His Asp Asp Phe Ile Gly Asn Gly Arg Val Gln Leu His Lys Val Leu
           85           90           95

Thr Arg Gly Tyr Asp Asp Ala Ser Trp Pro Leu Gln Thr Arg His Met
           100          105          110

Arg Ser Ala Gly Glu Val Thr Leu Ile Met His Phe Asp Val Ser Ala
           115          120          125

Met Lys Asn Lys Pro Gly Lys Ile Ser Ala Ala Ser Thr Thr His Ser
           130          135          140

Val Leu Pro Val Pro Val Pro Ala Val Pro Tyr Ala Ala Pro Ser Pro
           145          150          155          160

Ser Tyr Ala Leu Pro Pro Ala Gly Tyr Pro Ala Val Pro Pro Tyr Gln
           165          170          175

Ser Tyr Pro Ala Ser His Val Pro Ala Pro Tyr Pro Thr Ser Ala Tyr
           180          185          190

Pro His Pro Pro Pro Ser Leu Leu Ala Arg Asp Val Glu His Ala Ala
           195          200          205

Tyr Pro Pro Thr Ser Thr Thr Tyr Pro Pro Gln Pro Tyr Pro Pro Gln
           210          215          220

Pro Gln Gly Gln Thr Tyr Pro Pro Gln Pro Gln Gly Glu Thr Tyr Gln
           225          230          235          240

Pro Gln Pro Gln Arg Glu Thr Tyr Pro Pro Gln Pro Gln Val Gln Pro
           245          250          255

Tyr Pro Pro Lys Pro Gln Gly Gln Pro Tyr Pro Pro Gln Pro Gln Gly
           260          265          270

Gln Pro Tyr Pro Pro Gln Pro Tyr Gly Gln Thr Tyr Pro Pro Pro Pro
           275          280          285

Lys Gly Gln Pro Thr Tyr Pro Pro Ala Pro Tyr Pro Ser Thr Tyr Pro
           290          295          300

```

Pro Ala Pro Tyr
305

<210> 13
<211> 1172
<212> DNA
<213> Glycine max

<400> 13
ggtgaattgc aatttcaatt aattagaatt caacgtttgc aaattgcata ttgttcttct 60
cttcctctct tcctctgact ccatgtcgtc gataacgggc atccaggggc aacctcttga 120
ggttacggtg gtttcgtgct ccaagttgaa ggacacagaa tggatttcaa gacaagatcc 180
gtacgtttgt gttgagtatg gcagcacaaa gttccgaacc agaacctgca cagacggcgg 240
aaaaaacccg gtattccaag agaagttcat ctttcccctc attgaaggcc ttcgggagct 300
caatgtcctt gtttggaaaca gcaatactct caccttcgac gattttatag gaagcggaaa 360
gattcaattg cacaagggtc tctctcaagg ctctgatgac tctgcttggc cacttcagac 420
caaaaactggc agatacgtcg gtgaagtaaa agtcatattg cattacgcaa ttgcaaatca 480
aaggcataaa ttagtgctcag gccatgctcc atcagcacct ccgtatgtgg caacagcaac 540
tcctcccgtc ccttcttcat attctacttc ataccggcca cctccttctg ctacttccta 600
cccaccacca ccatcacctc cctctgcaac tccttaccat acaactggat cttattctta 660
cccaccggcg ccgccacctc ctacagctta ccctccctat tcctcacatt catctcccta 720
tcaccatca tcataccccc cacagccctc ctcgatcct cctcctcctc ccccatcatc 780
atatccccct gcttcagctt atccatatcc accacctgca ggctatcctt ctggaatata 840
ccctccacca ccttactgac tgagatcttc taccttctca accaaggaac caacatcaac 900
atgccttgta tgccaaaagg gccttcagac tccctttcaa tgcttggtca aacgccccgt 960
gctttgacct tttgaggtgt cttgcttgta aagtgtttat tttatacaca ttcagatcca 1020
attaaagggc accatttttt ttttcgcaat tggatgttca ctgaccattt tccgggtttc 1080
ttttgtctcc gtaaggatga aatatctatg aatcgtttat cagggttgctc aaaaaaaaaa 1140
aaaaaaaaac aaaaaaaaaa aaaaaaaaaa aa 1172

<210> 14
<211> 258
<212> PRT
<213> Glycine max

<400> 14
Met Ser Ser Ile Thr Gly Ile Gln Gly Gln Pro Leu Glu Val Thr Val
1 5 10 15
Val Ser Cys Ser Lys Leu Lys Asp Thr Glu Trp Ile Ser Arg Gln Asp
20 25 30
Pro Tyr Val Cys Val Glu Tyr Gly Ser Thr Lys Phe Arg Thr Arg Thr
35 40 45
Cys Thr Asp Gly Gly Lys Asn Pro Val Phe Gln Glu Lys Phe Ile Phe
50 55 60
Pro Leu Ile Glu Gly Leu Arg Glu Leu Asn Val Leu Val Trp Asn Ser
65 70 75 80
Asn Thr Leu Thr Phe Asp Asp Phe Ile Gly Ser Gly Lys Ile Gln Leu
85 90 95
His Lys Val Leu Ser Gln Gly Phe Asp Asp Ser Ala Trp Pro Leu Gln
100 105 110
Thr Lys Thr Gly Arg Tyr Ala Gly Glu Val Lys Val Ile Leu His Tyr
115 120 125

Ala Ile Ala Asn Gln Arg His Lys Leu Val Ser Gly His Ala Pro Ser
130 135 140

Ala Pro Pro Tyr Val Ala Thr Ala Thr Pro Pro Val Pro Ser Ser Tyr
145 150 155 160

Ser Thr Ser Tyr Pro Pro Pro Pro Ser Ala Thr Ser Tyr Pro Pro Pro
165 170 175

Pro Ser Pro Pro Ser Ala Thr Pro Tyr His Thr Thr Gly Ser Tyr Ser
180 185 190

Tyr Pro Pro Pro Pro Pro Pro Pro Thr Ala Tyr Pro Pro Tyr Ser Ser
195 200 205

His Ser Ser Pro Tyr Pro Pro Ser Ser Tyr Pro Pro Gln Pro Ser Ser
210 215 220

Tyr Pro Pro Pro Pro Pro Pro Ser Ser Tyr Pro Pro Ala Ser Ala Tyr
225 230 235 240

Pro Tyr Pro Pro Pro Ala Gly Tyr Pro Ser Gly Ile Tyr Pro Pro Pro
245 250 255

Pro Tyr

<210> 15
<211> 757
<212> DNA
<213> Zea mays

<400> 15
accacacgct cgcgccacgc gtccgcgcgc cgcgccgaag agaggagaga gcgcctccaa 60
cgccacctgg aggagaggac agcgcgccag ggagggggag gaggaagaag aacatgggga 120
agggcgctct gaaggtgcac ctctgtgcag ccaaggggt ctccggcaac gatttcttag 180
ggaagctgga cccctacgtg atcatgcagt accggagcca ggagcgcaag agcagcgctcg 240
cccgagacca aggaaggaa cgtgctgga acgaggtgtt caagtccag atcaactcgg 300
ccgcggccaa cgtgcagcac aagctcatcc tccggatcat ggaccacgac aacttctcca 360
gcgacgactt cctcggcgag gcgacgatcg acgtgacgga catcgtcagc ctgggcgcgcg 420
agcgcggcac gtaccacctc aacgcggcca agcacaacgt ggtcctcgcc gacaagacgt 480
accacggcga gatcaaggtc gccatcacct tcacctccac ccagaccag gttcaggaag 540
atggaggagc aattggagga tggaggcaca gtacgtttta tcaagttaa tgaaggcgt 600
cgtggactct ctcaagttct ttggttgctt ggtggtgttt cgggttggtat gtagtttttg 660
tttatgtcca cgagcaatct gtgcctaaca tttctagggt tcaattcaat gattcaatcc 720
aaccacacac aacacacac aacacacac aacacacac 757

<210> 16
<211> 157
<212> PRT
<213> Zea mays

<400> 16
Met Gly Lys Gly Val Leu Lys Val His Leu Val Asp Ala Lys Gly Leu
1 5 10 15

Ser Gly Asn Asp Phe Leu Gly Lys Leu Asp Pro Tyr Val Ile Met Gln
20 25 30

Tyr Arg Ser Gln Glu Arg Lys Ser Ser Val Ala Arg Asp Gln Gly Arg
 35 40 45
 Asn Pro Cys Trp Asn Glu Val Phe Lys Phe Gln Ile Asn Ser Ala Ala
 50 55 60
 Ala Asn Val Gln His Lys Leu Ile Leu Arg Ile Met Asp His Asp Asn
 65 70 75 80
 Phe Ser Ser Asp Asp Phe Leu Gly Glu Ala Thr Ile Asp Val Thr Asp
 85 90 95
 Ile Val Ser Leu Gly Ala Glu Arg Gly Thr Tyr His Leu Asn Ala Ala
 100 105 110
 Lys His Asn Val Val Leu Ala Asp Lys Thr Tyr His Gly Glu Ile Lys
 115 120 125
 Val Ala Ile Thr Phe Thr Ser Thr Gln Thr Gln Val Gln Glu Asp Gly
 130 135 140
 Gly Ala Ile Gly Gly Trp Arg His Ser Ser Phe Asn Gln
 145 150 155

<210> 17
 <211> 422
 <212> DNA
 <213> Hevea brasiliensis

<220>
 <221> unsure
 <222> (410)

<220>
 <221> unsure
 <222> (415)

<400> 17
 tcccaatcca cttgctcatt tcccttaagc tctatatata ccttttagaaa tttcttcttc 60
 ttgatctcca gaggtgtctt attcaatcct aaagcaagat tcaagaaacg gagatggcta 120
 ctgggctatt ggaagtgcag ctggtgaatg caaaaggcct cagaggcact gatttcttag 180
 gtaagattga tccatagtgt atcgtgaagt acaaaaacca agagcgcgag agcagtgtcg 240
 ccagaggtca aggtgggaat ccagtgtgga atgagaaact cacattcaag gtggaatatc 300
 cagggcaagg tgaagagtac aagctcattt taaaaatcat ggacaaggac accttctctg 360
 ctgatgattt gcttgggcca tgctacgata tatgtgaagg atttggtggn attangaatg 420
 ga 422

<210> 18
 <211> 102
 <212> PRT
 <213> Hevea brasiliensis

<220>
 <221> UNSURE
 <222> (99)

<220>
 <221> UNSURE
 <222> (101)

<400> 18

Met Ala Thr Gly Leu Leu Glu Val Gln Leu Val Asn Ala Lys Gly Leu
 1 5 10 15

Arg Gly Thr Asp Phe Leu Gly Lys Ile Asp Pro Tyr Val Ile Val Lys
 20 25 30

Tyr Lys Asn Gln Glu Arg Glu Ser Ser Val Ala Arg Gly Gln Gly Gly
 35 40 45

Asn Pro Val Trp Asn Glu Lys Leu Thr Phe Lys Val Glu Tyr Pro Gly
 50 55 60

Gln Gly Glu Glu Tyr Lys Leu Ile Leu Lys Ile Met Asp Lys Asp Thr
 65 70 75 80

Phe Ser Ala Asp Asp Leu Leu Gly His Ala Thr Ile Tyr Val Lys Asp
 85 90 95

Leu Leu Xaa Leu Xaa Met
 100

<210> 19

<211> 486

<212> DNA

<213> Glycine max

<220>

<221> unsure

<222> (430)

<220>

<221> unsure

<222> (464)

<220>

<221> unsure

<222> (486)

<400> 19

agaagaatag aatcttcaga gacatggcaa ttgggttcat ggaggtgcag cttgtgaaag 60
 caaaaggcct gcgagacact gatatctttg gtaaaatgga tccctatggt ctgatacaat 120
 acaaaggcca agagaagagg agtgggtgtcg ctaatggcaa aggcaaaaat cgggtatgga 180
 atgagaaatt tatcttcaaa gtagaatatc ctggatcaag caatcaacac aagctcatcc 240
 tcaaaattat ggataaagac ttatatacag acgacttcgt cggagaagca ataatccatg 300
 taggggattt attggcccaa ggagtagaga acggaggagc caaattacag actctcaagt 360
 atagagtgtg tcgtgctaac aagtcttatt gtgggtgaaat tgatgttggg tgttactttt 420
 accccgaaan gtgggaagac aaattttgtg ggaagaagac atangaggat ggaaaagaaa 480
 gtgacn 486

<210> 20

<211> 154

<212> PRT

<213> Glycine max

<220>

<221> UNSURE

<222> (136)

<220>

<221> UNSURE

<222> (147)

<400> 20

Met Ala Ile Gly Phe Met Glu Val Gln Leu Val Lys Ala Lys Gly Leu
 1 5 10 15

Arg Asp Thr Asp Ile Phe Gly Lys Met Asp Pro Tyr Val Leu Ile Gln
 20 25 30

Tyr Lys Gly Gln Glu Lys Arg Ser Gly Val Ala Asn Gly Lys Gly Lys
 35 40 45

Asn Pro Val Trp Asn Glu Lys Phe Ile Phe Lys Val Glu Tyr Pro Gly
 50 55 60

Ser Ser Asn Gln His Lys Leu Ile Leu Lys Ile Met Asp Lys Asp Leu
 65 70 75 80

Tyr Thr Asp Asp Phe Val Gly Glu Ala Ile Ile His Val Gly Asp Leu
 85 90 95

Leu Ala Gln Gly Val Glu Asn Gly Gly Ala Lys Leu Gln Thr Leu Lys
 100 105 110

Tyr Arg Val Val Arg Ala Asn Lys Ser Tyr Cys Gly Glu Ile Asp Val
 115 120 125

Gly Cys Tyr Phe Tyr Pro Glu Xaa Trp Glu Asp Lys Phe Cys Gly Lys
 130 135 140

Lys Thr Xaa Glu Asp Gly Lys Glu Ser Asp
 145 150

<210> 21

<211> 862

<212> DNA

<213> Glycine max

<400> 21

ttattagaca ttaaattgta agaattttgc tgacttgttaa gcttcagaga cgaagacaca 60
 cggtttagagt gagaaagaga tggcaattgg gtatcatggag gtgcagcttg tgaaagcaaa 120
 ggagtttgtt gacactgatt tctttggttag tatggacccg tatgttgtga tacaatacaa 180
 cggccaagag caaaggagta gtgttgctaa gggacagggc aataatccgg tatggaatga 240
 gaaatttgtg ttcaaggtag aatatcctac actgagtaat tcatacaaga ttatcttaaa 300
 aatcatggac aaggatcttt tatctgcaga tgactttgtt ggtcaagcca tagtctatgt 360
 ggaagatttta ttagccatag gggtagagga tgggtgcggct gagctacaac ctctaaagta 420
 cagagtaatt cgtgcagatc aatcttattg tggagaaatt gatcttggtg taacttttaa 480
 ggtggaagaa gagttcaatg gagaagctaa acgaggatcg aaggacagta aatagtattt 540
 gcaatagcag ttggccaaca tgaatatcaa ttgatttcaa tggagatttt ggaatcatca 600
 tcatgtagtt agtttcatct ttttagttgt atatgatcct tttggaaagt aggatcaatg 660
 catagataaaa ttactaaat tttatgccat caaattagta atagtatgca ttattaatct 720
 tctaatttat cttcaccata attaatctca ttgatgattc aatcttgtag ttccttaaca 780
 tctatatact atatgggttt gaacctttta aaaaaaagaa aaaaaaaaaa aaaaaaaaaa 840
 aaaaaaaaaa aaaaaaaaaa aa 862

<210> 22

<211> 151

<212> PRT

<213> Glycine max

<400> 22

Met Ala Ile Gly Phe Met Glu Val Gln Leu Val Lys Ala Lys Glu Leu
 1 5 10 15

Cys Asp Thr Asp Phe Phe Gly Ser Met Asp Pro Tyr Val Val Ile Gln
 20 25 30

Tyr Asn Gly Gln Glu Gln Arg Ser Ser Val Ala Lys Gly Gln Gly Asn
 35 40 45

Asn Pro Val Trp Asn Glu Lys Phe Val Phe Lys Val Glu Tyr Pro Thr
 50 55 60

Leu Ser Asn Ser Tyr Lys Ile Ile Leu Lys Ile Met Asp Lys Asp Leu
 65 70 75 80

Leu Ser Ala Asp Asp Phe Val Gly Gln Ala Ile Val Tyr Val Glu Asp
 85 90 95

Leu Leu Ala Ile Gly Val Glu Asp Gly Ala Ala Glu Leu Gln Pro Leu
 100 105 110

Lys Tyr Arg Val Ile Arg Ala Asp Gln Ser Tyr Cys Gly Glu Ile Asp
 115 120 125

Leu Gly Ile Thr Phe Lys Val Glu Glu Glu Phe Asn Gly Glu Ala Lys
 130 135 140

Arg Gly Ser Lys Asp Ser Lys
 145 150

<210> 23

<211> 860

<212> DNA

<213> Triticum aestivum

<400> 23

tccaaacgcg acctcatcag agcaagaccc ggaggaaaca aggagaggcc agagcggcct 60
 gtcacaaggc aaaggacaga ggaggtgctt gttcaggtct cctgctagat ccggaggcga 120
 tgggcagggg cgtgctggag gtgcatctcg tcgacgccaa gggcctcttc ggcagcgatt 180
 tcctagggaa gatcgaccgc tatgtaatcg tgcaataccg gagccaggag cgcaagagca 240
 gcacctccag agatgagggg aggaaccoga gctggaacga ggtgttccgg ttccagatca 300
 actcctctgc ggccaacggg cagcacaagc tcttctctcc gatcatggac cagcacaact 360
 tctccagcga cgacttctc ggccaagcga cgatcaacgt gaccgatctg atcagcaccg 420
 gcatggagag cggcgcgtcg cagctgaacg cggcaaagta cagcgttgtg tccgctgata 480
 actcatacca cggcgagatc agagtaggcc tcacgttcac cgccaccaag gttgaagaag 540
 acggagggca ggtcggaggc tggacgcaca gctotcgaga gtagagcatg taacgtcctt 600
 gcccttcgct cgtagcttta gtgttgatg ctatgatgtc cgtgactgaa tgatgtgatt 660
 ccaagtgtat gtacgttgca cctgtagtag ctttttagaa gatgtatatg tactagtagc 720
 cagaagtcag aactcgtagc aggctagagg cgtcaattcc gttaattaat tgtcgatttg 780
 tgggtcttat tttaggggga attgtgattc tggatgcgaa caccaaaaaa aaaaaaaaaa 840
 aaaaaaaaaa aaaaaaaaaa 860

<210> 24

<211> 154

<212> PRT

<213> Triticum aestivum

<400> 24

Met Gly Arg Gly Val Leu Glu Val His Leu Val Asp Ala Lys Gly Leu
 1 5 10 15

Phe Gly Ser Asp Phe Leu Gly Lys Ile Asp Pro Tyr Val Ile Val Gln
 20 25 30

Tyr Arg Ser Gln Glu Arg Lys Ser Ser Thr Ser Arg Asp Glu Gly Arg
 35 40 45

Asn Pro Ser Trp Asn Glu Val Phe Arg Phe Gln Ile Asn Ser Ser Ala
 50 55 60

Ala Asn Gly Gln His Lys Leu Phe Leu Arg Ile Met Asp His Asp Asn
 65 70 75 80

Phe Ser Ser Asp Asp Phe Leu Gly Gln Ala Thr Ile Asn Val Thr Asp
 85 90 95

Leu Ile Ser Thr Gly Met Glu Ser Gly Ala Ser Gln Leu Asn Ala Ala
 100 105 110

Lys Tyr Ser Val Val Ser Ala Asp Asn Ser Tyr His Gly Glu Ile Arg
 115 120 125

Val Gly Leu Thr Phe Thr Ala Thr Lys Val Glu Glu Asp Gly Gly Gln
 130 135 140

Val Gly Gly Trp Thr His Ser Ser Arg Glu
 145 150

<210> 25

<211> 914

<212> DNA

<213> Oryza sativa

<400> 25

cttttggaag aaaagatcac caaaaaccct atattccata gttgagacac aagatttttt 60
 gaagccaagt ttgcgcattha catcaaaggg ttcttttgat gcgaccaatg ctgtgaagag 120
 tgtaactagc agtatctcta gcgcttcagg gaagcatgtc gctgacgata caagagaatt 180
 tgttgagag ctgaacatta cagtggtaag aggtattcag ttggccgtca gagacatgct 240
 aacgagcgat ccataatgtt ttctaacact tggggagcag aaagctcaaa ccactgttaa 300
 accgagtgc ttgaaccacg tatggaatga ggtgcttaag atatcaattc ctcgaaatta 360
 tggacctctt aaacttgaag tatacgacca tgatacgttc tctgctgatg atatcatggg 420
 ggaagcggag atagatcttc aaccaatgat cacagccgtc atggcctttg gagatccctc 480
 gcgtgttggg gacatgcaaa ttggaagggtg gttcatgacc aaagacaatg ccctggtgaa 540
 agatagcact gtcaatgttg tgcggggcaa ggtaaaacag gaagtgcacc taaagttaga 600
 gaatgtagaa tcaggtgaga tggagttaga actggaatgg gttccaatac cctagattaa 660
 taaagctcga ttggttctct gccaaaaaaa attactcaag aagcgtcagt tttgtaattt 720
 aaatgaatgg cttcaaatcc cgtgtactta ctgaatctct gtcttcaaca ttttggccac 780
 ccgaacgaaa ttcgtaaaaa tgccattgta aaatatcatg ttgtaatccg tcggctgcac 840
 tcacgaccaa ttatattatt ctttagtgaa gtgtgctttc aaccctgtgt cataaaaaaa 900
 aaaaaaaaaa aaaa 914

<210> 26

<211> 217

<212> PRT

<213> Oryza sativa

<400> 26

Phe Trp Lys Lys Arg Ser Pro Lys Thr Leu Tyr Ser Ile Val Glu Thr
 1 5 10 15

Gln Asp Phe Leu Lys Pro Ser Leu Arg Ile Thr Ser Lys Gly Ser Phe
 20 25 30

Asp Ala Thr Asn Ala Val Lys Ser Val Thr Ser Ser Ile Ser Ser Ala
 35 40 45

Ser Gly Lys His Val Ala Asp Asp Thr Arg Glu Phe Val Gly Glu Leu
 50 55 60

Asn Ile Thr Val Val Arg Gly Ile Gln Leu Ala Val Arg Asp Met Leu
 65 70 75 80

Thr Ser Asp Pro Tyr Val Val Leu Thr Leu Gly Glu Gln Lys Ala Gln
 85 90 95

Thr Thr Val Lys Pro Ser Asp Leu Asn Pro Val Trp Asn Glu Val Leu
 100 105 110

Lys Ile Ser Ile Pro Arg Asn Tyr Gly Pro Leu Lys Leu Glu Val Tyr
 115 120 125

Asp His Asp Thr Phe Ser Ala Asp Asp Ile Met Gly Glu Ala Glu Ile
 130 135 140

Asp Leu Gln Pro Met Ile Thr Ala Val Met Ala Phe Gly Asp Pro Ser
 145 150 155 160

Arg Val Gly Asp Met Gln Ile Gly Arg Trp Phe Met Thr Lys Asp Asn
 165 170 175

Ala Leu Val Lys Asp Ser Thr Val Asn Val Val Ser Gly Lys Val Lys
 180 185 190

Gln Glu Val His Leu Lys Leu Gln Asn Val Glu Ser Gly Glu Met Glu
 195 200 205

Leu Glu Leu Glu Trp Val Pro Ile Pro
 210 215

<210> 27

<211> 770

<212> DNA

<213> Oryza sativa

<400> 27

ccacgcgtcc ggccctgtgca acatcatcat caagaagaag aagagatcaa cggcaagaag 60
 actagcgact agcgagagat cgatcgaaga gaagaggaga gatggtgcac gggaagctgg 120
 aggtcctcct cgtctgcgcc aagggcctcg aggacactga cttcttgaac gacatggacc 180
 cctacgtgat cctcaactgc cgcactcagg agcagaaaag cagcgttgca aaaggagcag 240
 gaagcgagcc tgaatggaac gagaccttgc tcttcaccgt ctccgacgat gttccacagc 300
 tcaatgtcaa gatcatggac agtcatgact tctcagctga cgatttcgtc ggtgaagcaa 360
 acattcctct ggagcctgtg ttccctggaag gcagccttcc tccagccgtc caccgtgtcg 420
 tcaaggagga gaagtactgt ggagagatca aggttgctct cacccttact ccagcagcgg 480
 aaactcgcca tcatcacaac caccgagaac agggggaggg ttacagcagc tggaactgat 540
 tgcctgtctac taatgagcat caacgagagg agatcttgtc tcaagaatta atgtgcttgt 600
 caacaatact ccgtgctatg atgtcctaag aactgaaaca tccatttata tgtatatccc 660

agaccattga cttgctctgc ctaaattttg tatatttttt actacaaaga tgtgatggtg 720
 tgaaatccag aatattttta tcgaaaaaaa aaaaaaaaaa aaaaaaaaag 770

<210> 28
 <211> 145
 <212> PRT
 <213> Oryza sativa

<400> 28
 Met Val His Gly Lys Leu Glu Val Leu Leu Val Cys Ala Lys Gly Leu
 1 5 10 15
 Glu Asp Thr Asp Phe Leu Asn Asp Met Asp Pro Tyr Val Ile Leu Thr
 20 25 30
 Cys Arg Thr Gln Glu Gln Lys Ser Ser Val Ala Lys Gly Ala Gly Ser
 35 40 45
 Glu Pro Glu Trp Asn Glu Thr Phe Val Phe Thr Val Ser Asp Asp Val
 50 55 60
 Pro Gln Leu Asn Val Lys Ile Met Asp Ser Asp Ala Phe Ser Ala Asp
 65 70 75 80
 Asp Phe Val Gly Glu Ala Asn Ile Pro Leu Glu Pro Val Phe Leu Glu
 85 90 95
 Gly Ser Leu Pro Pro Ala Val His Arg Val Val Lys Glu Glu Lys Tyr
 100 105 110
 Cys Gly Glu Ile Lys Val Ala Leu Thr Phe Thr Pro Ala Ala Glu Thr
 115 120 125
 Arg His His His Asn His Glu Asn Glu Gly Glu Gly Tyr Ser Ser Trp
 130 135 140

Asn
 145

<210> 29
 <211> 958
 <212> DNA
 <213> Glycine max

<400> 29
 gcacagaaag aaaaaagttg gatccagcca aattccagct ccaatttgta actcactgct 60
 tcaggcattt ctggcacaat tttttccacc tttatttcaa ctttaagact ccacagaaag 120
 aagcatattc ctgagtcaaa tagttctgtc catatagaat ttgtgaagtg agagtccaac 180
 ctttcatttt caattttcaa agatgcctcg tggaacactt gaagttgttc tgatcagcgc 240
 caaaggaatc gatgacaatg attttctctc cagcatagat ccttatgtga ttctcacata 300
 cagggcacag gagaaaaaga gcaactgtgca agaagatgct ggatccaagc cacaatggaa 360
 tgagagcttt cttttcactg tctctgacag tgcttctgaa cttaatctga agataatgga 420
 taaagacaac tttagtcaag atgattgtct tggcgaggca accattcatt tagatccagt 480
 gtttgaagcc ggtagcattc cagaaactgc ttacaagggt gtgaaggacg aagaatattg 540
 tggtagattt aaggtggctc tcactttcac tgctgagaga aatgaggagc agggttatga 600
 tgcacctgaa gagagctatg gtggatggaa agaattccagt ggggaatatt aaagtgaag 660
 aagaatttac atacttcaat ggccagactt acctttataa tgaaaaataa gcagttttgg 720
 tgtcactctt aggcatttc cattattgtg ttttctggtg tgaagatcca atagtgttg 780
 gcttttaggt tgcattcctc ctttggata ttaaagtaca ttatgcttga tatattatct 840

tttatgcatc agttaaacat tagaagagca gtgctatattt atttataaaaa aaaaaaaaaa 900
 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 958

<210> 30
 <211> 149
 <212> PRT
 <213> Glycine max

<400> 30
 Met Pro Arg Gly Thr Leu Glu Val Val Leu Ile Ser Ala Lys Gly Ile
 1 5 10 15
 Asp Asp Asn Asp Phe Leu Ser Ser Ile Asp Pro Tyr Val Ile Leu Thr
 20 25 30
 Tyr Arg Ala Gln Glu Lys Lys Ser Thr Val Gln Glu Asp Ala Gly Ser
 35 40 45
 Lys Pro Gln Trp Asn Glu Ser Phe Leu Phe Thr Val Ser Asp Ser Ala
 50 55 60
 Ser Glu Leu Asn Leu Lys Ile Met Asp Lys Asp Asn Phe Ser Gln Asp
 65 70 75 80
 Asp Cys Leu Gly Glu Ala Thr Ile His Leu Asp Pro Val Phe Glu Ala
 85 90 95
 Gly Ser Ile Pro Glu Thr Ala Tyr Lys Val Val Lys Asp Glu Glu Tyr
 100 105 110
 Cys Gly Glu Ile Lys Val Ala Leu Thr Phe Thr Ala Glu Arg Asn Glu
 115 120 125
 Glu Gln Gly Tyr Asp Ala Pro Glu Glu Ser Tyr Gly Gly Trp Lys Glu
 130 135 140
 Ser Ser Gly Glu Tyr
 145

<210> 31
 <211> 695
 <212> DNA
 <213> Triticum aestivum

<400> 31
 gcacgaggag agatccaaga ctaggccggc cggccggagg agatcgagaa ggaggaggag 60
 acatggtgcg cgggaagctg gaggtgctgc tcgtctccgc caagggcctc gacgactccg 120
 atttcttcaa tagcatggac ccgtacgtga tcctcacctg ccgcagccac gaggagaaga 180
 gcaccgtcgc atcaggagca gggagcagag ctgagtggaa cgagaccttc gtcttcgccg 240
 tctccggcga cgctccggag ctccaggtca agatcatgga cagcgacgcc ctctcggccg 300
 acgacctcgt cggagaagca tgtatcccgc tggaggctgt gctccaggag ggcagcctgc 360
 cgccggccgt gcaccgggtc gtcaaggacg aggagtaccg cggggagatc aagatcgcg 420
 tcaccttcac cccggcagag gaaaacgagg aggaggagga gagctacggc ggctggaatc 480
 agtccacctg aaaaaggcca gcgagccagc aagatggtgc tgtatgtctg actgtcataa 540
 tggatagaaa ggctttggat atccttgatg tgtgtgacag acagggcatt caggaaaacg 600
 agtaaaaata ggggaaatat gtatcgatgc atgcatgaag tactaatcaa gcaattcacc 660
 gcatcgtttt gtattgcaaa aaaaaaaaaa aaaaaa 695

<210> 32
 <211> 142

<212> PRT

<213> Triticum aestivum

<400> 32

Met Val Arg Gly Lys Leu Glu Val Leu Leu Val Ser Ala Lys Gly Leu
 1 5 10 15

Asp Asp Ser Asp Phe Phe Asn Ser Met Asp Pro Tyr Val Ile Leu Thr
 20 25 30

Cys Arg Ser His Glu Gln Lys Ser Thr Val Ala Ser Gly Ala Gly Ser
 35 40 45

Glu Pro Glu Trp Asn Glu Thr Phe Val Phe Ala Val Ser Gly Asp Ala
 50 55 60

Pro Glu Leu Arg Val Lys Ile Met Asp Ser Asp Ala Leu Ser Ala Asp
 65 70 75 80

Asp Leu Val Gly Glu Ala Cys Ile Pro Leu Glu Ala Val Leu Gln Glu
 85 90 95

Gly Ser Leu Pro Pro Ala Val His Arg Val Val Lys Asp Glu Glu Tyr
 100 105 110

Arg Gly Glu Ile Lys Ile Ala Leu Thr Phe Thr Pro Ala Glu Glu Asn
 115 120 125

Glu Glu Glu Glu Glu Ser Tyr Gly Gly Trp Asn Gln Ser Thr
 130 135 140

<210> 33

<211> 617

<212> DNA

<213> Zea mays

<220>

<221> unsure

<222> (421)

<400> 33

cacgccgcct ccatgtgggt ggggaggcaa acgcgttcgt ccatctctga aactcaaacg 60
 ccttgattg gagcatacta caggagtact tctgtacaaa tataaataacc cctggcgagt 120
 tgggttgggt ctatctcgca atcgaggcgt ttttttctg cttcgtaagt tcgtgggcga 180
 tccagcgagc gagcgagcag accggcggcc aaccgcggag ggagagatgg cgcaggggac 240
 gctggagggt cttctcgctg gagccagggg cctcgagaac accgattacc tgagcaacat 300
 ggacccttac gcgcttctgc aatgtcgctc ccacgagcag aagagcagcg tcgcatctgg 360
 caaaggctgt gaacctgagt ggaacgagac cttcgtgttc accgtctcca accggcgaca 420
 ngagctgttc atcaagctcc tggacagtga cggtggcact gatgacgatt ttgttggtga 480
 agcaacgatt cctctggaag ccagtttaca cggaaggaa gcattccttc cgactgttta 540
 caatgttgtg aaagacgaag aataccgcgg agaaatcaaa gttggcctca cgttcactcc 600
 agaggtaaac catctca 617

<210> 34

<211> 202

<212> PRT

<213> Zea mays

<220>

<221> UNSURE

<222> (140)

<400> 34

Thr Pro Pro Pro Cys Gly Trp Gly Gly Lys Arg Val Arg Pro Ser Leu
 1 5 10 15

Lys Leu Lys Arg Leu Val Leu Glu His Thr Thr Gly Val Leu Leu Tyr
 20 25 30

Lys Tyr Lys Tyr Pro Trp Arg Val Gly Leu Gly Leu Ser Arg Asn Arg
 35 40 45

Gly Val Phe Phe Leu Leu Arg Lys Phe Val Val Asp Pro Ala Ser Glu
 50 55 60

Arg Ala Asp Arg Arg Pro Thr Ala Glu Gly Glu Met Ala Gln Gly Thr
 65 70 75 80

Leu Glu Val Leu Leu Val Gly Ala Arg Gly Leu Glu Asn Thr Asp Tyr
 85 90 95

Leu Ser Asn Met Asp Pro Tyr Ala Leu Leu Gln Cys Arg Ser His Glu
 100 105 110

Gln Lys Ser Ser Val Ala Ser Gly Lys Gly Cys Glu Pro Glu Trp Asn
 115 120 125

Glu Thr Phe Val Phe Thr Val Ser Asn Gly Ala Xaa Glu Leu Phe Ile
 130 135 140

Lys Leu Leu Asp Ser Asp Gly Gly Thr Asp Asp Asp Phe Val Gly Glu
 145 150 155 160

Ala Thr Ile Pro Leu Glu Ala Ser Leu His Gly Lys Glu Ala Phe Leu
 165 170 175

Pro Thr Val Tyr Asn Val Val Lys Asp Glu Glu Tyr Arg Gly Glu Ile
 180 185 190

Lys Val Gly Leu Thr Phe Thr Pro Glu Val
 195 200

<210> 35

<211> 544

<212> DNA

<213> Zea mays

<220>

<221> unsure

<222> (415)

<220>

<221> unsure

<222> (478)

<220>

<221> unsure

<222> (494)

<220>
 <221> unsure
 <222> (509)

 <220>
 <221> unsure
 <222> (515)

 <220>
 <221> unsure
 <222> (531)..(532)

<220>
 <221> unsure
 <222> (542)

<400> 35
 gttcgttcac gccacaggca aggcacaggg gcttgtgagg gagagcgagg agcggaggag 60
 gacatgggtgc acgggacgct ggaagtgtg ctcgttgggg ccaagggcct cgagaacacc 120
 gattacctct gtaacatgga tccgtatgca attctcaagt gccgttcaca ggagcagaag 180
 agcagtattg caactggaaa aggaactacc cctgagtgga atgaaaactt tatcttcact 240
 gtgtctgacc ggacaacaga cttggtaatc aagcttatgg acagtgatac aggcacagca 300
 gatgactttg ttggtgaagc aacgattcca ttggaagcag tgtatactga aaggagcatt 360
 ccaccaacac tctataatgt tgtgaaaggt gaaaaatact gcgggggaaat caaantgggtc 420
 tcacattcac tcctgaggat actcgcaagc gggctctcaa aggacttcgt ggtggaanca 480
 tcatcttaag ctantcttta gggtcacana cacancacaa tcatcgcttg nncctcacog 540
 tnat 544

<210> 36
 <211> 130
 <212> PRT
 <213> Zea mays

<220>
 <221> UNSURE
 <222> (118)

<400> 36
 Met Val His Gly Thr Leu Glu Val Leu Leu Val Gly Ala Lys Gly Leu
 1 5 10 15
 Glu Asn Thr Asp Tyr Leu Cys Asn Met Asp Pro Tyr Ala Ile Leu Lys
 20 25 30
 Cys Arg Ser Gln Glu Gln Lys Ser Ser Ile Ala Thr Gly Lys Gly Thr
 35 40 45
 Thr Pro Glu Trp Asn Glu Asn Phe Ile Phe Thr Val Ser Asp Arg Thr
 50 55 60
 Thr Asp Leu Val Ile Lys Leu Met Asp Ser Asp Thr Gly Thr Ala Asp
 65 70 75 80
 Asp Phe Val Gly Glu Ala Thr Ile Pro Leu Glu Ala Val Tyr Thr Glu
 85 90 95
 Arg Ser Ile Pro Pro Thr Leu Tyr Asn Val Val Lys Gly Glu Lys Tyr
 100 105 110

Cys Gly Glu Ile Lys Xaa Gly Leu Thr Phe Thr Pro Glu Asp Thr Arg
 115 120 125

Lys Arg
 130

<210> 37
 <211> 459
 <212> DNA
 <213> Triticum aestivum

<220>
 <221> unsure
 <222> (435)

<400> 37
 gccgagcttt ccatttttca actcctagtc ctatacatatc agcgggaaccc cgggggctcgg 60
 atcggatcta cagcaattag tctcgacctt cagtcgtgcc gcctgctcat cagcatataa 120
 ttcctgatcg agcagcgagg agaggaaggg gagatcaggc cgggagagaa gatggcgag 180
 gggacgctgg aggtgctgct cgtgggagcc aagggcctcg agaacaccga ctacctctgc 240
 aacatggacc cgtacgcggt tctaaaatgc acctcgacagg agcaaaaagag caccgtcgcc 300
 tctggaaagg gaagtgatcc tgagtgaac gaaacctttg tgttcaccgt ctctgagaat 360
 gcaactgagc ttgtcatcaa gctactggac agtggatggt gcacggacga cgacagcggt 420
 ggtgaagcaa cgatncattg gatggagtgt acactgaag 459

<210> 38
 <211> 87
 <212> PRT
 <213> Triticum aestivum

<400> 38
 Met Ala Gln Gly Thr Leu Glu Val Leu Leu Val Gly Ala Lys Gly Leu
 1 5 10 15

Glu Asn Thr Asp Tyr Leu Cys Asn Met Asp Pro Tyr Ala Val Leu Lys
 20 25 30

Cys Thr Ser Gln Glu Gln Lys Ser Thr Val Ala Ser Gly Lys Gly Ser
 35 40 45

Asp Pro Glu Trp Asn Glu Thr Phe Val Phe Thr Val Ser Glu Asn Ala
 50 55 60

Thr Glu Leu Val Ile Lys Leu Leu Asp Ser Asp Gly Gly Thr Asp Asp
 65 70 75 80

Asp Ser Val Gly Glu Ala Thr
 85

<210> 39
 <211> 417
 <212> DNA
 <213> Oryza sativa

<400> 39
 atcgtcaact cagctcctct cttttcttccc ctcccccgct cctccgcgag acgacccgcg 60
 cccgtagcca tccatgtcga tacaaggcca gatcctcgaa gtcagagtca ctgggtgcag 120
 gaagctgagg gacacggagt tcttcacggc gcaggatccc tacgtctgca tcgagtatgc 180
 caccaacaag ttccgcaccc gcacctgcac cgatggggga aggaacccta cttttgacga 240
 gaagtttcat atacctctca ttgaggggct tcgtgagcta accgtcacag tgtggaacag 300

caacacgctc acccatgatg atttcattgg caatggcagg gtgcaagctg cataaggtgc 360
 ttacgcgtgg ctatgatgat gcctcaaggg cctccagac acgccatattg aggtctg 417

<210> 40
 <211> 83
 <212> PRT
 <213> Oryza sativa

<400> 40
 Leu Glu Val Arg Val Thr Gly Cys Arg Lys Leu Arg Asp Thr Glu Phe
 1 5 10 15
 Phe Thr Arg Gln Asp Pro Tyr Val Cys Ile Glu Tyr Ala Thr Asn Lys
 20 25 30
 Phe Arg Thr Arg Thr Cys Thr Asp Gly Gly Arg Asn Pro Thr Phe Asp
 35 40 45
 Glu Lys Phe His Ile Pro Leu Ile Glu Gly Leu Arg Glu Leu Thr Val
 50 55 60
 Thr Val Trp Asn Ser Asn Thr Leu Thr His Asp Asp Phe Ile Gly Asn
 65 70 75 80
 Gly Arg Val

<210> 41
 <211> 550
 <212> DNA
 <213> Glycine max

<220>
 <221> unsure
 <222> (534)

<400> 41
 ggtgaattgc aatttcaatt aattagaatt caacgtttgc aaattgcata ttgttcttct 60
 cttcctctct tctctgact ccattgtcgtc gataacgggc atccagggcc aacctcttga 120
 gggttacgggt gtttcgtgct ccaagttgaa ggacacagaa tggatttcaa ggcaagatcc 180
 gtacgtttgt gttgagtatg gcagcacaaa gttccgaacc agaacctgca cagacggcgg 240
 aaaaaatccg gtattccaag agaagttcat cttcccccctc attgaaggcc ttcggggagct 300
 caatgtcctt gtttggaaca gcaatactct cacettggac gattttatag gaagcggaaa 360
 gattcaattg cacaagggtc tctctcaagg cttcgatgac tctgcttggc cacttcagac 420
 caaaactggc agatacgctg gtgaagtcaa agtcatattg cattacgcaa ttgcaaatca 480
 tcaaaggcat aaatcagtggt caagccatgc tccatcaaca cctccgtatg tggnaacaac 540
 aactcctccc 550

<210> 43
 <211> 424
 <212> DNA
 <213> Zea mays

<220>
 <221> unsure
 <222> (169)..(170)

<220>
 <221> unsure
 <222> (172)..(173)

<220>
 <221> unsure
 <222> (178)..(179)..(180)

<220>
 <221> unsure
 <222> (183)

<400> 43
 acccacgcgt ccgcccacgc gtccgcgcgcg ccgccgcaag agaggagaga ggcgcctccaa 60
 cgccacctgg aggagaggac agcgcgccag ggagggggag gaggaagaag aacatgggga 120
 agggcgctct gaaggtgcac ctcgctcgacg ccaagggggt ctccggcann gnnttctnnn 180
 ggnagctgga cccctacgtg atcatgcagt accggagcca ggagcgcaag agcagcgctcg 240
 ccgagacca aggaaggaac ccgtgctgga acgaggtgtt caagttccag atcaactcgg 300
 ccgcggccaa cgtgcagcac aagctcatcc tccggatcat ggaccacgac aacttctcca 360
 gcgacgactt ctcggcgagg cgacgatoga cgtgacggac atcgtcagcc tgggcgcca 420
 gcgc 424

<210> 44
 <211> 85
 <212> PRT
 <213> Zea mays

<220>
 <221> UNSURE
 <222> (18)..(19)

<220>
 <221> UNSURE
 <222> (21)..(22)..(23)

<400> 44
 Gly Lys Gly Val Leu Lys Val His Leu Val Asp Ala Lys Gly Leu Ser
 1 5 10 15
 Gly Xaa Xaa Phe Xaa Xaa Xaa Leu Asp Pro Tyr Val Ile Met Gln Tyr
 20 25 30
 Arg Ser Gln Glu Arg Lys Ser Ser Val Ala Arg Asp Gln Gly Arg Asn
 35 40 45
 Pro Cys Trp Asn Glu Val Phe Lys Phe Gln Ile Asn Ser Ala Ala Ala
 50 55 60
 Asn Val Gln His Lys Leu Ile Leu Arg Ile Met Asp His Asp Asn Phe
 65 70 75 80
 Ser Ser Asp Asp Phe
 85

<210> 45
 <211> 548
 <212> DNA
 <213> Glycine max

<220>
 <221> unsure
 <222> (291)

<220>
 <221> unsure
 <222> (349)

<220>
 <221> unsure
 <222> (417)

<220>
 <221> unsure
 <222> (437)

<220>
 <221> unsure
 <222> (446)

<220>
 <221> unsure
 <222> (486)

<220>
 <221> unsure
 <222> (492)

<220>
 <221> unsure
 <222> (506)

<220>
 <221> unsure
 <222> (525)

<220>
 <221> unsure
 <222> (528)

<220>
 <221> unsure
 <222> (544)

<400> 45
 ttaaattgta agaattttgc tgacttgtaa gcttcagaga cgaagacaca cggtttagagt 60
 gagaaagaga tggcaattgg gttcatggag gtgcagcttg tgaaagcaaa ggagtttgt 120
 gacactgatt tctttggtag tatggaccgc tatgttgtga tacaatacaa cggccaagag 180
 caaaggagta gtgttgctaa gggacagggc aataatccgg tatggaatga gaaatttgtg 240
 ttcaaggtag aatatcctac actgagtaat tcatacaaga ttatcttaaa natcatggac 300
 aaggatcttt tatctgcaga tgactttggt ggtcaagcca tagtcctang tgggaagatt 360
 tattagccat aaggggtaga ggatgggtgcc ggctgagcta caacctccta aagtacnaga 420
 gtaattccgt gcagatnaat ccttantggg ggagaaattg atcttgggat aacttttaaa 480
 gggggnaaga angagttcaa tggagnaagc ctaaaccaag gatcnaangg acagtaaatt 540
 agtntttc 548

<210> 46
 <211> 89
 <212> PRT
 <213> Glycine max

<220>
 <221> UNSURE
 <222> (71)

<400> 46

Gly Phe Met Glu Val Gln Leu Val Lys Ala Lys Glu Leu Cys Asp Thr
 1 5 10 15

Asp Phe Phe Gly Ser Met Asp Pro Tyr Val Val Ile Gln Tyr Asn Gly
 20 25 30

Gln Glu Gln Arg Ser Ser Val Ala Lys Gly Gln Gly Asn Asn Pro Val
 35 40 45

Trp Asn Glu Lys Phe Val Phe Lys Val Glu Tyr Pro Thr Leu Ser Asn
 50 55 60

Ser Tyr Lys Ile Ile Leu Xaa Ile Met Asp Lys Asp Leu Leu Ser Ala
 65 70 75 80

Asp Asp Phe Val Gly Gln Ala Ile Val
 85

<210> 47

<211> 473

<212> DNA

<213> Triticum aestivum

<220>

<221> unsure

<222> (296)

<220>

<221> unsure

<222> (473)

<400> 47

tccaaacgcg acctcatcag agcaagaccc ggaggaaaca aggagaggcc agagcggcct 60
 gtcacaaggc aaggacagag gaggtgcttg ttcaggcttc ctgctagatc cggaggcgat 120
 gggcaggggc tgctggaggt gcatctcgtc gacgccaaagg gcctcttcgg cagcgatttc 180
 ctagggaagat cgacccgtat gtaatcgtgc aataccggag ccaggagcgc aagagcagca 240
 ctccagagat gaggggagga acccgagctg gaacgaggtg ttccggttcc agatcnctcc 300
 tctgcggcca acgggcagca caagctcttc ctccggatca tggaccacga catcttctcc 360
 agcgacgact tctcggcca agcgacgac aacgtgaccg atctgatcag accggcatgg 420
 agaagcgggc gcgtcgcagc tgaacgcggc aaagtacaac gttgttgtcc gcn 473

<210> 48

<211> 99

<212> PRT

<213> Triticum aestivum

<220>

<221> UNSURE

<222> (24)

<220>

<221> UNSURE

<222> (47)

<220>

<221> UNSURE

<222> (62)

<400> 48

Gly Gln Gly Leu Leu Glu Val His Leu Val Asp Ala Lys Gly Leu Phe
 1 5 10 15

Gly Ser Asp Phe Leu Gly Arg Xaa Asp Pro Tyr Val Ile Val Gln Tyr
 20 25 30

Arg Ser Gln Glu Arg Lys Ser Ser Thr Pro Glu Met Arg Gly Xaa Gly
 35 40 45

Glu Glu Pro Glu Leu Glu Arg Gly Val Pro Val Pro Asp Xaa Ser Ser
 50 55 60

Ala Ala Asn Gly Gln His Lys Leu Phe Leu Arg Ile Met Asp His Asp
 65 70 75 80

Ile Phe Ser Ser Asp Asp Phe Leu Gly Gln Ala Thr Ile Asn Val Thr
 85 90 95

Asp Leu Ile

<210> 49

<211> 465

<212> DNA

<213> Oryza sativa

<400> 49

aaagatcacc caaaacccta tattccatag ttgagacaca agattttttg aagccaagtt 60
 tgcgcattac atcaaagggt tcttttgatg cgaccaatgc tgtgaagagt gtaactagca 120
 gtatctctag cgcttcaggg aagcatgtcg ctgacgatac aagagaattt gttggagagc 180
 tgaacattac agtggttaaga ggtattcaag ttggccgtca gagacatgct aacgagcgat 240
 ccatatgttg ttctaact tggggagcag aaagctcaaa ccactgttaa accgagtgc 300
 ttgaaccag tatggaatga ggtgcttaag atatcaattc ctcgaaatta tggacctctt 360
 aaacttgaag tatacgacca tgatacgttc tctgctgatg atatcatggg ggaagcggag 420
 atagatcttc aaccaatgat cacagccgctc atggcctttg gagaa 465

<210> 50

<211> 31

<212> PRT

<213> Oryza sativa

<400> 50

Val Val Leu Thr Leu Gly Glu Gln Lys Ala Gln Thr Thr Val Lys Pro
 1 5 10 15

Ser Asp Leu Asn Pro Val Trp Asn Glu Val Leu Lys Ile Ser Ile
 20 25 30

<210> 51

<211> 390

<212> DNA

<213> Oryza sativa

<220>

<221> unsure

<222> (43)

<220>
 <221> unsure
 <222> (204)

<220>
 <221> unsure
 <222> (301)

<220>
 <221> unsure
 <222> (347)

<220>
 <221> unsure
 <222> (373)

<400> 51
 gcctgtgcaa catcatcatc aagaagaaga agagatcaac ggnaagaaga ctacgcgacta 60
 gcgagagatc gatcgaagag aagaggagag atggtgcacg ggaagctgga ggtcctcctc 120
 gtctgcgcca agggcctcga ggacactgac ttcttgaacg acatggaccc ctacgtgatc 180
 ctcacctgcc gcactcagga gcangaaaag cagcgttgca aaaggagcag gaagcgagcc 240
 tgaatggaac gagaccttcg tcttcaccgt ctcgcacgat gttccacagc tcaatgtcaa 300
 ngatcatgga caagtgatgg ccttctcaag ctgacgattt cggtcnnggt gaagcaaaca 360
 attcctctgg gangcctgtg ttccctggaa 390

<210> 52
 <211> 69
 <212> PRT
 <213> Oryza sativa

<400> 52
 Met Val His Gly Lys Leu Glu Val Leu Leu Val Cys Ala Lys Gly Leu
 1 5 10 15
 Glu Asp Thr Asp Phe Leu Asn Asp Met Asp Pro Tyr Val Ile Leu Thr
 20 25 30
 Cys Arg Thr Gln Glu Gln Lys Ser Ser Val Ala Lys Gly Ala Gly Ser
 35 40 45
 Glu Pro Glu Trp Asn Glu Thr Phe Val Phe Thr Val Ser Asp Asp Val
 50 55 60
 Pro Gln Leu Asn Val
 65

<210> 53
 <211> 489
 <212> DNA
 <213> Glycine max

<220>
 <221> unsure
 <222> (417)

<220>
 <221> unsure
 <222> (428)

<220>
 <221> unsure
 <222> (452)

<220>
 <221> unsure
 <222> (482)

<400> 53
 agaaagaaaa aagtggatcc agccaaattc cagctccaat ttgtaactca ctgcttcagg 60
 catttctggc acaatttttt ccacctttat ttcaacttta agactccaca gaaagaagca 120
 tattcctgag tcaaatagtt ctgtccatat agaatttgtg aagtgagagt ccaacctttc 180
 attttcaatt ttcaaagatg cctcgtggaa cacttgaagt tgttctgac agcgccaaag 240
 gaatcgatga caatgatttt ctctccagca tagatcotta tgtgattctc acatacaggg 300
 cacaggagaa aaagagcact gtgcaagaaa gatgctggat ccaagccaca atggaatgag 360
 agctttcttt tctactgtctc tgacagtgtc tctgaactta atctgaagat aatgggntaa 420
 agacaacntt agtcaaagat ggttggcctg gngaggggaac caatcaatta gattcaagtg 480
 gnttggaggg 489

<210> 54
 <211> 42
 <212> PRT
 <213> Glycine max

<400> 54
 Met Pro Arg Gly Thr Leu Glu Val Val Leu Ile Ser Ala Lys Gly Ile
 1 5 10 15
 Asp Asp Asn Asp Phe Leu Ser Ser Ile Asp Pro Tyr Val Ile Leu Thr
 20 25 30
 Tyr Arg Ala Gln Glu Lys Lys Ser Thr Val
 35 40

<210> 55
 <211> 523
 <212> DNA
 <213> Triticum aestivum

<220>
 <221> unsure
 <222> (401)

<220>
 <221> unsure
 <222> (407)

<220>
 <221> unsure
 <222> (449)

<220>
 <221> unsure
 <222> (456) .. (457)

<220>
 <221> unsure
 <222> (493)

<220>
 <221> unsure
 <222> (497)

<220>
 <221> unsure
 <222> (512)

<400> 55
 gagagatcca agactaggcc ggccgggccg aggagatcga gaaggaggag gagacatggt 60
 gcgcgggaag ctggagggtgc tgctcgtctc cgccaagggc ctcgacgact ccgatttctt 120
 caatagcatg gaccgcgtacg tgatcctcac ctgccgcagc cacgagcaga agagcaccgt 180
 cgcattcagga gcagggagcg agcctgagtg gaacgagacc ttctgtcttcg ccgtctccgg 240
 cgacgctccg gagctcaggg tcaagatcat ggacagcgac gccctctcgg ccgacgacct 300
 cgtcggagaa gcatgtatcc cgctggaggc tgtgctccag gagggcagcc tgccgccggc 360
 cgtgcaccgg gtctcaagga cgaggagtac cgccggggaat naagatngcg ctcaactcac 420
 ccggcagagg aaaacaggag gaggaggana ctacgnnggt ggatcatcac tgaaaaggca 480
 cgagcacaaa tngtntttt acgtaaaagg anaaagggtt gat 523

<210> 56
 <211> 28
 <212> PRT
 <213> Triticum aestivum

<400> 56
 Met Val His Gly Lys Leu Glu Val Leu Leu Val Ser Ala Lys Gly Leu
 1 5 10 15
 Glu Asp Thr Asp Phe Leu Asn Asn Met Asp Pro Phe.
 20 25

WO 00/60088

PCT/US00/09110

<220>
 <221> unsure
 <222> (497)

<220>
 <221> unsure
 <222> (512)

<400> 55
 gagagatcca agactaggcc ggccggccgg aggagatcga gaaggaggag gagacatggt 60
 gcgcgggaag ctggaggtgc tgctcgtctc cgccaagggc ctcgacgact ccgatttctt 120
 caatagcatg gacccgtacg tgatcctcac ctgccgcagc cacgagcaga agagcaccgt 180
 cgcacagga gcagggagcg agcctgagtg gaacgagacc ttcgtcttcg ccgtctccgg 240
 cgacgctccg gagctcaggg tcaagatcat ggacagcgac gccctctcgg ccgacgacct 300
 cgtcggagaa gcatgtatcc cgctggaggc tgtgctccag gagggcagcc tgccgccggc 360
 cgtgcaccgg gtctcaagga cgaggagtac cgcggggaat naagatngcg ctcacttcac 420
 ccggcagagg aaaacaggag gaggaggana ctacgnnggt ggatcatcac tgaaaaggca 480
 cgagcacaaa tgngttnttt acgtaaaagg anaaagggtt gat 523

<210> 56
 <211> 28
 <212> PRT
 <213> Triticum aestivum

<400> 56
 Met Val His Gly Lys Leu Glu Val Leu Leu Val Ser Ala Lys Gly Leu
 1 5 10 15
 Glu Asp Thr Asp Phe Leu Asn Asn Met Asp Pro Phe
 20 25